



Response of mineral formulation towards different growth phases of arabica coffee in lowland

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Abstract

Arabica coffee cultivation is limited by altitude, which affects its production. Some farmers in Jember Regency are innovating to grow arabica coffee in the lowland, but the potential for leaf rust disease is quite high. Plant natural resistance can be enhanced by adding minerals formulation (containing silica, iodine, and calcium). This research aimed to determine which phase is more effective for applying mineral formulation that induces plant resistance. The formulation was tested on arabica coffee plants grown at the lowland (460 masl) in Jember Regency on 12 years old (y.o) mature and 1 y.o immature plants. One formulation was dissolved in 14 L of water and applied by foliar feeding. The effect was analyzed using two-way T-test of two samples assuming unequal variances at 95% confidence level to determine the differences. The analysis showed that the plants supplemented with the formulation could increase the variables that supported the natural resistance of plants (both mechanically and through the production of chemical compounds), such as polyphenol content, flavonoids, antioxidant activity, total dissolved protein, vitamin C, reducing sugar, lignin, cellulose, hemicellulose, and iodine content in the plant. The response of the formulation application showed that the most resistance variables was better in 1 y.o immature plants than in 12 y.o mature plants. The application of mineral formulation in immature phase will have a better impact on increasing natural resistance, and it has the potential to be used as a supplement for arabica coffee plants cultivated in the lowland.

INTRODUCTION

Indonesia is a country that has the largest coffee cultivation area in ASEAN (Association of South East Asia Nations) countries covering land area of 1.238.596 Ha (Triyanti, 2016). The high trade in arabica coffee in the international market has caused the demand for arabica coffee to continue to increase. The result of Kustiari (2007) reported that arabica coffee trade in International market reached 70%, while Indonesia could only produce arabica coffee about 189.740 tons/year (Kementerian Pertanian, 2019). The low productivity of Indonesia arabica coffee is partly due

to the limited cultivation land in the upland (above 1000 meters above sea level) to reduce the impact of leaf rust disease.

Some farmers have innovated cultivating arabica coffee in the lowland area to increase the productivity of Indonesian arabica coffee. However, the potential of leaf rust attack is higher in the lowland area, and the natural resistance of the plant must be increased. Rosyady et al. (2020) mentions that mineral elements such as silica, iodine, and potassium can increase the plant resistance to attack by biotrophic fungi, such as *H. vastatrix*. Furthermore, Wulanjari et al. (2020) also applied mineral element to coffee plants affected

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by leaf rust disease, and it showed decreasing in incidence by up to 78,13%. Based on the result of this study, these mineral elements have the potential to be formulated as supplements that can increase the natural resistance of plants in arabica coffee that have not been attacked by leaf rust disease. By applying mineral elements, arabica coffee grown in the lowland is expected to be more resistant to leaf rust. Coffee plants have two growth stages, namely immature and mature plants. Photosynthate in immature plants focuses on vegetative growth, such as hardening the stem, branch, and twigs. The coffee plants in this stage are more susceptible to leaf rust disease. According to the research of Ibrahim et al. (2017), the natural severity of leaf rust disease in 10 coffee seedlings tested species reached 25%, with an incidence of more than 60%. The photosynthate in the mature phase focuses on forming the coffee bean. Pani et al. (2023) stated that the incidence of leaf rust in the mature stage in Aceh Province was in the range of 40–51%, while the study of Sugiarti (2017) reported the severity of leaf rust attack in experimental field in the range of 30–90%. However, the research has yet to be tested on different plant phases. Thus, further test is necessary to find out when the mineral formulation is more effective to be given for increasing the natural resistance of arabica coffee plants cultivated in lowlands.

MATERIALS AND METHODS

This research was conducted from August to December 2020 on 1-year-old immature and 12-year-old mature arabica coffee plants grown in the NV. Kalianda Concern Jember Regency at an altitude of about 460 meters above sea level (lowland). The mineral fertilizer formulation was tested on two different coffee plant phases, immature and mature plants, with ten replications. Every formulation packaging contains 43.49 grams of mineral powder (silica, iodine, and calcium) to dissolve in 14 liters of water. The formulation was applied once by foliar feeding technique. To allow the formulation to get integrated with plant metabolism, the plants were incubated for a period of 14 days following treatment. After incubation, leaf samples were air dried and dried using an oven for three days at 70°C. Then the samples were powdered for analysis preparation. The analysis was carried out in the laboratory to determine several chemical compounds, including (1) polyphenol content using the Folin Ciocalteu method with gallic acid

equivalent (Xue et al., 2016), (2) flavonoid content using colorimetry method with quercetin equivalent (Xue et al., 2016), (3) antioxidant activity using DPPH extract (Siswoyo et al., 2011), (4) total dissolved protein using the Bradford method (Xue et al., 2016), (5) vitamin C using spectrophotometry (Xue et al., 2016), (6) reducing sugars using the DNS (Dinitrosalysilic Acid) method (Pratiwi et al., 2018), (7) hemicellulose, lignin, and cellulose using the Chesson method (Kanani et al., 2018), and (8) iodine content using the spectrophotometry. The data were analyzed using two-way T-test of two samples assuming unequal variances at 95% confidence level to determine the effects caused by treatment.

RESULTS AND DISCUSSION

The research findings showed that a formulation containing three minerals could raise the levels of antioxidant activity, protein, vitamin C, reducing sugar, hemicellulose, lignin, cellulose, and iodine in plants as well as their polyphenol and flavonoid content. However, the amounts varied depending on the growth phase of the plant. The application of formulation in one-year-old immature arabica coffee plants presented a better effect than that applied to the twelve-year-old mature plants, as shown by the analysis using two-way T-test of two samples assuming unequal variances (Table 1). The T-test revealed a significant difference at a 95% significance level in the polyphenol content, flavonoids, total dissolved protein, reducing sugar, hemicellulose, lignin, and iodine content. However, no significant difference was observed in the antioxidant activity, vitamin C, and leaves cellulose content.

Plant maturity level is indicated by plant age. Young plants behave differently when treated with mineral formulations because their metabolisms differ from mature plants. Similarly, the level of resistance was found in both young and old plants' leaves. Numerous substances in coffee leaves, including steroids, terpenoids, flavonoids, and polyphenols, are capable of having antioxidant functions (Hasanah et al., 2017). Coffee leaves' flavonoid, polyphenol, and antioxidant content might be used as an indicator of plant resistance. Flavonoids contain antioxidant activities that assist oxidative stress and prevent or treat various kinds of diseases. The study conducted by Subroto et al. (2019) explains the correlation between flavonoid content and the resistance of

Table 1. T-test result in various resistance variables due to addition of mineral formulation

Observed variable	Unit	Average in one-year-old immature plants	Average in 12-year-old mature plants	T stat	T table	Result of T-test*
Polyphenol	mg GAE/g	27.01	18.13	3.24	2.120	Significant
Flavonoid	mg QE/g	27.03	18.13	3.24	2.120	Significant
Antioxidant activity	%	59.46	57.16	1.99	2.160	Insignificant
Total dissolved solid	mg/g	2.74	4.28	9.16	2.120	Significant
Vitamin C	mg/g	3.96	4.13	0.44	2.101	Insignificant
Sugar reduction	mg/g	47.16	38.29	2.42	2.131	Significant
Hemicellulose	%	9.59	8.00	2.16	2.131	Significant
Lignin	%	26.72	30.63	3.31	2.120	Significant
Cellulose	%	24.65	24.97	0.25	2.110	Insignificant
Iodine content	%	0.02	0.03	6.77	2.101	Significant

Remarks: Based on T stat compared with two-way T-table at 95% confidence level. Critical value of two ways T-table at $\alpha = 5\%$ ranges from -2.1009 to 2.1009.

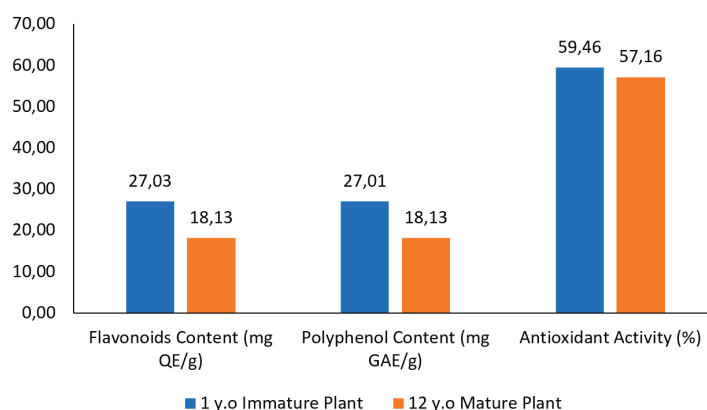


Figure 1. Flavonoid content, polyphenol content, and antioxidant activity in arabica coffee leaves

arabica coffee plants against leaf rust disease. Compared to the mature phase of the plant, which is 12 years old, the addition of mineral formulations to a 1-year-old immature plant resulted in increased flavonoid content, polyphenol content, and antioxidant activity, reaching 27.027 mg GAE/g; 27.005 mg QE/g; and 59.46%, respectively (Figure 1). Flavonoid production in the immature phase regulates cell growth (Dias et al., 2021). The growth of the cell tends to be faster in the vegetative phase than in the other phases. Cell damage during the vegetative period is the main cause of the synthesis of compounds such as flavonoids, polyphenols and antioxidants. Calcium of the mineral formulation forms calcium pectate in the middle lamella, which may limit damage to cells, that can lead to an increase in flavonoid production. It causes the flavonoids

formed in immature plants to be identified higher, followed by the production of polyphenols and antioxidants, the largest antioxidant components in plants (Djapiala et al., 2013). Mature plants produce more flavonoids to help them resist stress, however, in this research, the plants were not facing any stress. As a result, there was less synthesis of antioxidants, polyphenols, and flavonoids. Figure 1 generally represents the number of polyphenols, flavonoids, and antioxidant activity in arabica coffee leaves after the application of mineral formulation.

An antioxidant works to both prevent and restore cell damage. The main content of an antioxidant in coffee leaves are ethyl acetate, N-hexane, and water fractions. Hasanah et al. (2017) stated that the fraction with the highest antioxidant activity in coffee leaves

was ethyl acetate fraction with an IC_{50} reaching 37.07 ppm. Vitamin C levels in leaf tissue also reveal particular antioxidants. When it comes to scavenging free radicals, vitamin C has similarities to antioxidants. As a result, vitamin C is commonly used as the reference. When plants get disease, vitamin C is essential for their capacity to recover. Plants with higher vitamin C content may be better to recover. When formulation was added to mature arabica coffee plants that were 12 years old, the vitamin C content increased (4.13 mg/g) compared to the one-year-old immature plants (3.96 mg/g); however, the T-test did not detect a significant difference in this variable.

The presence of 0.02% iodine in the leaf tissue of the one-year-old immature plants also contributed to the increased polyphenol content. Mature coffee leaves showed higher iodine content than one-year-old immature plants, which was 0.03%, but produced lower polyphenols. Reduced levels of polyphenols indicate that the 0.02 percent iodine in one-year-old

immature plant might have a phytotoxic effect, causing mild stress as the plant increases the synthesis of polyphenols (Li et al., 2017). Iodine can boost plant tolerance to stress by increasing the synthesis of antioxidants, such as polyphenols, SOD, COD, and peroxidase. Iodine, at the optimum concentration, can suppress the production of catalase and MDA (malondialdehyde/lipid peroxidase product), which can damage the plant cell membranes. However, when the concentration is higher than ideal, it can cause the peroxidase to destroy the cell membranes. The synthesis of lignocellulosic components, such as lignin, cellulose, and hemicellulose, is also increased by the higher polyphenol content found in plant tissue. As affected by the application of mineral-based formulation, the hemicellulose content of arabica coffee leaves in the immature plant was higher than in the mature plant phase (Figure 2). This is due to the fact that young plants generate more polyphenols than mature plants do, while a high concentration

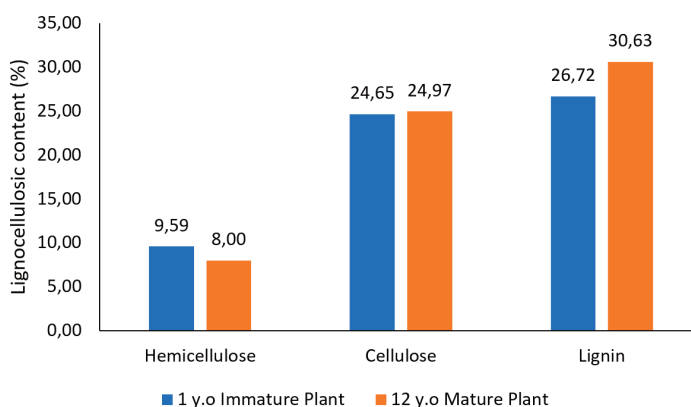


Figure 2. Lignocellulosic content in arabica coffee leaves after application of formulation

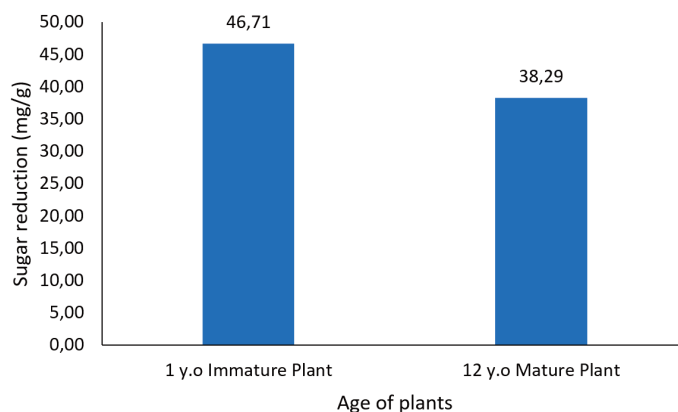


Figure 3. Sugar reduction content in arabica coffee leaves after application of formulation

of polyphenols in mature plants can result in the formation of phenolic polymers, which take the form of lignocellulosic materials (Martoharsono, 1994). This result is supported by several studies, stating that in leaf tissue, the lignocellulosic material is dominated by hemicellulose (Barokah et al., 2018; Nurnasari and Nurindah 2018; Wulanjari et al., 2022). Other lignocellulosic materials, such as lignin, produced significantly different and higher values in the 12-year-old mature plant (30.63%) than one-year-old immature plants (26.72%) (Figure 2) due to the difference in the age of mature plants that were older than immature plants. The cell in mature plants tend to lead to cell wall thickening, so they have lower water content, and the cell walls become thicker and stiffer (Keraf et al., 2015). Mature leaves have a larger lignin concentration due to the higher rate of lignification. Additionally, it could increase plant biomass. Likewise, the cellulose content of the 12-year-old mature coffee plants was higher (24.96%) than that of the one-year-old immature plants (24.64%) despite the insignificant result of the statistical test. Additionally, the addition of silica and calcium from the formulation, which are deposited on the cell wall, supports the lignocellulosic material content. Calcium combines with pectin to form calcium pectate, while silica associates with cellulose to form silica cellulose. The addition of mineral formulations was observed in mature plants as cellulose and lignin forms.

The addition of mineral formulation resulted in a higher protein content in the mature plants. According to Keraf et al. (2015), when the plants are more mature, the water content is lower, and the total dissolved protein content is also lower, leading to higher crude/rough fiber. The result of this experiment is contrary to the result of the research by Keraf et al. (2015) presumably because the mature plant used was an arabica plant that was 12 years old. The age of the plant is the golden age, where healthy plants will produce high productivity, so the protein content is also higher than protein content in the one-year-old immature plant.

Another crucial factor that shows the plant's potential for natural resistance is reducing sugar. Since reduced sugar is present in diseased plants, pests can utilize it as a substrate or food source. Adding formulation to one-year-old arabica coffee plant gives a higher reducing sugar content (Figure 3). The elements of silica and calcium in mineral formulations increase the resistance of plant cells through the

formation of calcium-pectate in the middle lamella and silica-cellulose in the outer cell wall, thereby causing the cells to become stronger. The rate of photosynthesis in healthy young plants is faster than in mature plants, so it also requires higher energy. Reducing sugar acts as a respiration substrate to produce energy. The higher reduced sugar in healthy plants will result in higher energy for growth, and this energy is also used for self-defense when pests attack the plants.

CONCLUSIONS

The mineral formulation inducing natural resistance of lowland arabica coffee was more effective when applied in one-year-old immature plants, as indicated by the significant differences in the content of polyphenol, flavonoids, reducing sugar, and hemicellulose. The antioxidant activity produced by one-year-old immature plants was higher although statistically not significantly different. The protein, lignin, and iodine content tend to be higher in the 12-year-old mature plants. The content of vitamin C and cellulose was also higher in 12-year-old mature plants, but not significantly different from one-year-old immature plants.

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